J. Iran. Chem. Soc., Vol. 7, No. 4, December 2010, pp. 883-889.

JOURNAL OF THE
Iranian
Chemical Society

Enzymatic Resolution of Racemic Sulcatol by Lipase from *Candida Antarctica* in a Large Scale

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(Received 10 July 2009, Accepted 29 January 2010)

Large scale enzymatic resolution of racemic sulcatol **2** has been useful for stereoselective biocatalysis. This reaction was fast and selective, using vinyl acetate as donor of acyl group and lipase from *Candida antarctica* (CALB) as catalyst. The large scale reaction (5.0 g, 39 mmol) afforded high optical purities for *S*-(+)-sulcatol **2** and *R*-(+)-sulcatyl acetate **3**, *i.e.*, *ee* > 99 per cent and good yields (45 per cent) within a short time (40 min). Thermodynamic parameters for the chemoesterification of sulcatol **2** by vinyl acetate were evaluated. The enthalpy and Gibbs free energy values of this reaction were negative, indicating that this process is exothermic and spontaneous which is in agreement with the reaction obtained enzymatically.

Keywords: Enzymatic resolution, Lipase, *Candida antarctica*, Sulcatol, Biocatalysis

INTRODUCTION

Natural sulcatol **2** is a male aggregation pheromone of the ambrosia beetle, *Gnathrotrichus sulcatus*. The enantiomeric composition of the natural chiral pheromone was determined by NMR spectroscopy as 30 percent *ee* for (*S*)-(+)-sulcatol **2**. Both enantiomers are required for bioactivity. Enantiopure (*S*)- and (*R*)-sulcatol **2** are not bioactives, and the racemic mixture of sulcatol **2** was more active than the natural pheromone [1]. The bioactivity of many chiral compounds depends on the main composition of one of the enantiomers. Therefore, methods to synthesize pure enantiomers are constantly required. At present, biocatalysis is an important methodology for the synthesis of enantiomerically pure compounds [2-5]. Several enzymatic methodologies for the synthesis of sulcatol **2** using lipases are described in the literature. For example: lipase PS from *Pseudomonas cepacia* (*E* = 10-101) [6] and *(E* = 26-101) [7], *Pseudomonas* sp. (*E* = 27-29) [8], lipase Amano PS (*E* = 11-38) [9-10], lipase PPL from *Pseudomonas* sp. (*E* = 32-44) [11], lipase PPL Sigma (*E* = 17-62) [12], lipase PPL Sigma [(*S*)-sulcatol **2** (*ee* > 98 per cent) and (*R*)-sulcatol **2** (*ee* > 61 per cent)] [13], lipase PPL (*E* = 19-100) [14], lipase from *Burkholeria cepacia*-Amano PS (*E* = 29 -40) [15], lipase from *Nigella sativa* (*E* = 2.9-4.1) [16].

Enzyme catalyzed esterifications of sulcatol **2** afforded good or modest selectivities by using various groups of acyl donors in different solvents and experimental conditions. A convenient enzymatic kinetic resolution using CALB was obtained for sulcatol **2** on a small scale, using *i*-Pr_2O as solvent for 5 h (*E* = 183) [17].

Enzymatic hydrolysis of (±)-sulcatyl acetate **3** by lipase Novozyme 435 produced sulcatol in high excess (*ee* > 99

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percent, $E > 300$) while the unreacted sulcatyl was obtained in poor selectivity (*ee* 48 percent) [18]. The (±)-sulcatyl acetate **3** was initially hydrolyzed with lipase PS from *Pseudomonas* cepacia, and 93 percent *ee* (*R*)-sulcatol **2** was obtained. In addition, the (*R*)-**2** was acetylated into (*S*)-**3** and subjected to a second hydrolysis with lipase PS to give 100 percent *ee* (*R*)-sulcatol 2 [19].

The elegant preparation of chiral sulcatol (*R*)-**2** (ee >98 percent) was obtained by lipase-catalyzed deracemization of (±)-**2** using kinetic resolution coupled with an *in situ* inversion or, alternatively, dynamic resolution using combined lipase and Ru-catalysis [20]. The preparation of (*S*)-sulcatol **2** and (*R*)-sulcatyl acetate **3** on a small scale (35 mg $mmol^{-1}$) has been described as CALB lipase, and chiral products were obtained with high enantiomeric excesses (> 98 percent *ee*) [21].

Biological reductions of sulcatone **1** have been described for the preparation of sulcatol **2** in good enantiomeric excesses (*ee* > 80-99 percent) [22-23]. The efficient non-enzymatic methodology of chemical kinetic resolution of (±)-sulcatol **2** using chiral aldehyde/$In(OTf)_3$ produced (*R*)-sulcatol **2** in high enantiomeric excess [24].

Enantioselectivities were expressed as enantiomeric ratio (*E*), from the degree of conversion and the corresponding enantiomeric excess value of the product by means of equations developed by Chen and Sih [25].

In this work, we describe in detail the enzymatic resolution of sulcatol **2**, on a small (40.0 mg) and a large scale (5.0 g), using lipase from *Candida antarctica* (CALB). To our knowledge, no methodology affords both enantiomers with high enantiomeric purities (*ee* > 98 percent, $E > 200$), within a short time period (40 min), high performance (yield = 45 percent) and on a large scale, *e.g.*, 5.0 g of sulcatol (±)-**2** by enzymatic resolution.

EXPERIMENTAL

General Methods

Reagents (vinyl acetate, sulcatone, anhydride and pyridine) and solvents (ethyl acetate and hexane) were purchased from Sigma-Aldrich (St. Louis, MO, USA) and Synth (São Paulo, SP, Brazil). Tecnal TE-421 (São Paulo, SP, Brazil) or Superohm G-25 (Piracicaba, SP, Brazil) orbital shakers were employed in the biocatalysed reactions. Purification of the reaction products was carried out by column chromatography (CC) over silicagel (230-400 mesh) eluted with mixtures of *n*-hexane and ethyl acetate (9:1; 8:2). The collected fractions were monitored by TLC on aluminium-backed pre-coated silicagel 60 F254 layers eluted with hexane and ethyl acetate (8:2), and visualized by spraying with *p*-anisaldehyde/ sulphuric acid reagent followed by heating at *ca*. 120 °C. Reaction products were analyzed using a Hewlett Packard (Palo Alto, CA, USA) model HP-5890 gas chromatograph, equipped with a flame ionisation detector (FID) and a Varian (Palo Alto, CA, USA) Chiral CP-Chiralsil-DEX (β-Cyclodextrin) column (25 m × 0.25 mm i.d.; 0.39 μm). The chromatographic conditions were: oven temperature initially at 50 °C (2 min) and increased to 100 °C (1 min) at 2 °C min^{-1}, injector temperature 200 °C; detector temperature 200 °C; injector split ratio 1:20; hydrogen carrier gas at a pressure of 60 kPa; and run time 32.5 min. The enantiomeric excess (*ee*) of sulcatol **2** and sulcatyl acetate **3** were determined by GC analysis in which the retention time of *S*-(**2**) was 21.7 min and of *R*-(**2**) was 22.4 min; of *S*-(**3**) was 21.3 and of *R*-(**3**) was 23.9 min. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AC-200 (^{1}H: 500 MHz; ^{13}C: 125 MHz) spectrometer using $CDCl_3$ as solvent. Near IR spectra were obtained on a Bomen MB-102 spectrometer. Novozyme 435, immobilized lipase from *Candida antartica*, was provided by NovoNordisk (Araucária, Paraná-PR, Brazil).

Synthesis of (±)-Sulcatol 2

Racemic sulcatol **2** was prepared by reducing the corresponding sulcatone **1** using sodium borohydride in methanol. Sulcatone **1** (630.5 mg, 5 mmol), $NaBH_4$ (80 mg, 2 mmol) and methanol (10 ml) were added to a 25 ml flask equipped with a magnetic stirrer. The reaction mixture was stirred for 1 h at room temperature. After that, the reaction was quenched by the addition of water (1 ml), the methanol was removed under vacuum and the residue was extracted with ethyl acetate (3 × 20 ml). The combined organic phases were dried over magnesium sulfate and then filtered. The organic solvent was evaporated under reduced pressure and the residue purified by silicagel column chromatography using hexane/ethyl acetate (9:1; 8:2) as eluent to produce racemic alcohol **2** in excellent yield (595.8 mg, 93 per cent). The

spectroscopy data (^{1}H NMR, MS and IR) of **3** agreed with the data reported in the literature [26].

Synthesis of (±)-Sulcatyl Acetate 3

Alcohol **2** was acetylated using classical conditions to obtain the corresponding racemic acetate **3**. Sulcatol **2** (512.48 mg, 4 mmol), pyridine (0.5 ml) and acetic anhydride (0.5 ml) were added to a 25 ml flask equipped with a magnetic stirrer. The reaction mixture was stirred for 24 h at room temperature. The reaction was quenched by the addition of 10 percent HCl (2 ml), and the organic phase extracted with ethyl acetate (3 × 20 ml). The combined organic phases were dried over magnesium sulfate and then filtered. The organic solvent was evaporated under reduced pressure and the residue purified by silicagel column chromatography using hexane/ethyl acetate (9:1; 8:2) as eluent to produce racemic acetate **3** in excellent yield (555 mg, 90 percent). 6-Methylhept-5-en-2-yl acetate (**3**): yield, 90 percent; yellow oil. The spectroscopy data (^{1}H NMR, ^{13}C NMR, MS and IR) of **3** agreed with the data reported in the literature [27].

Small Scale Biocatalyzed Enzymatic Reaction

Racemic sulcatol **2** (40 mg, 0.31 mmol) was added to a 50 ml Erlenmeyer flask containing 10 ml of hexane (HPLC grade), 0.5 ml of vinyl acetate and 80 mg of CALB. The reaction mixture was stirred in an orbital shaker (32 °C, 150 rpm) until the consumption of the reagents (Table 1). After that, the mixture was filtered and the solvent evaporated. The residue was purified by silicagel column chromatography using hexane/ethyl acetate (9:1) as eluent to produce sulcatol (*S*)-**2** and acetate (*R*)-**3** in excellent yields (Table 1).

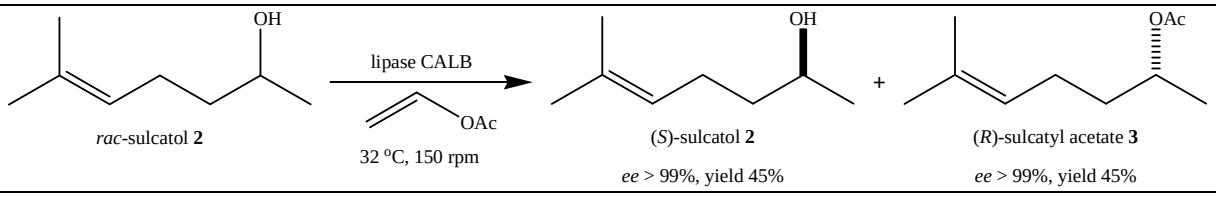
Large Scale Biocatalyzed Enzymatic Reaction

A similar procedure to the one in small scale was adopted in a large scale reaction. In this case, a 125 ml Erlenmeyer flask was used containing 20 ml of hexane, 2.0 ml of vinyl acetate, 350 mg of CALB and 5.0 g (39 mmol) of racemic sulcatol **2** (Table 1).

Control of the Enzymatic Resolution of Sulcatol 2 by Lipase

The progress of the reactions was monitored (Table 1) by collecting samples (0.1 ml) which were analyzed by GC/FID (1 µl) in a chiral capillary column. The products of the biocatalyzed reactions were compared with the previously analyzed racemic mixtures.

Table 1. Small and Large Scale Chemoenzymatic Esterification of (±)-Sulcatol **2** Using CALB Lipase



rac-sulcatol **2** | (*S*)-sulcatol **2** *ee* > 99%, yield 45% | (*R*)-sulcatyl acetate **3** *ee* > 99%, yield 45%

t[d]	c (%)[a] **2**	*ee* (%)[b] **2**	AC[h] **2**	Yield (%)[e] **2**	$[\alpha]_D^{25}$ **2**	c (%)[a] **3**	*ee* (%)[b] **3**	AC[h] **3**	Yield (%)[e] **3**	$[\alpha]_D^{25}$ **3**	*E*[c]
20[f]	52	48	*S*	-	-	48	99	*R*	-	-	-
40[f]	50	99	*S*	-	-	50	99	*R*	-	-	-
60[f]	50	99	*S*	-	-	50	99	*R*	-	-	-
120[f]	50	99	*S*	45	-	50	99	*R*	45	-	-
120[g]	50	99	*S*	45	+ 10.5° (*c* 0.4, $CHCl_3$)	50	99	*R*	45	+ 3.8° (*c* 0.4, $CHCl_3$)	>200

[a]c = conversion. The conversion rate [c = $ee_s/(ee_s + ee_p)$] was ~50 percent in all reactions. [b]*ee* = enantiomeric excesses determined by chiral GC. [c]The enantiomeric ratio (*E*) was calculated using Sih's method [25]. [d]t = time (min). [e]Yield isolated. [f]Small scale. [g]Large scale. [h]Absolute configuration.

Assignment of the Absolute Configuration

The optical rotation of the products from the biocatalytic reaction was measured in a Perkin-Elmer (Waltham, MA, USA) model 241 polarimeter using a 1 dm cuvette and referenced to the Na-D line. The absolute configuration of (*S*)-(+)-**2** was determined comparing the specific rotation sign measured for the product with that reported in the literature [9,20,22,24]. The absolute configuration of sulcatyl acetate (*R*)-(+)- **3** was in full agreement with that previously reported [26]. *S*-(+)-6-Methylhept-5-en-2-ol (**2**): $[\alpha]^{25}_{D}$ + 10.5° (*c* 0.4, $CHCl_3$), *ee* > 98 percent and *R*-(+)-6-methylhept-5-en-2-yl acetate (**3**): $[\alpha]^{25}_{D}$ + 3.8° (*c* 0.4, $CHCl_3$), *ee* > 98 percent.

Theoretical Methods

The electronic calculations were carried out using the GAUSSIAN 03 software [28]. Stationary points on the potential energy surface of the reaction system were fully optimized, and the harmonic vibration frequencies evaluated to characterize their nature as minima or first-order saddle points; the absence of imaginary frequencies indicated that all optimized structures were true minima. We used Becke's three-parameter hybrid functionals, which include a mixture of HF exchange with DFT exchange-correlation. The DFT method was applied in this case employing the B3LYP functional [29] that uses the non-local correlation provided by the LYP expression [30-31]. Calculations were made using the B3LYP/6-311++G(d,p)//B3LYP/6-31G(d,p) method. The gas-phase enthalpy and Gibbs free energy (at 1 atm) were calculated as follows:

$$\Delta_R H^o_{298K} = \Sigma_{Products}[\Delta_f H^o_{prod}(298\ K)] - \Sigma_{reactants}[\Delta_f H^o_{react}(298\ K)]$$

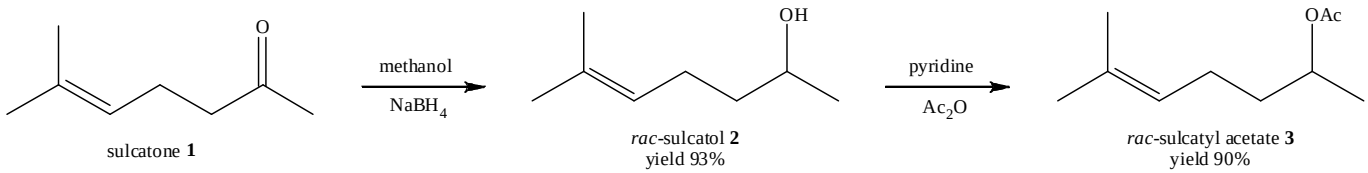
$$\Delta_R G^o_{298K} = \Sigma_{Products}[\Delta_f G^o_{prod}(298\ K)] - \Sigma_{reactants}[\Delta_f G^o_{react}(298\ K)]$$

RESULTS AND DISCUSSIONS

Racemic sulcatol **2** was synthesized in excellent yield through the reduction of the corresponding sulcatone **1**, using sodium borohydride in methanol (Scheme 1). Sulcatyl acetate **3** was synthesized through the esterification of sulcatol **2,** employing the classical methodology, using acetic anhydride in the presence of pyridine (Scheme 1). The racemic sulcatol **2** was submitted to CALB-catalyzed enzymatic acetylation with vinyl acetate as the acyl donor. In other experiments, we have used this reagent, which has shown excellent performance for this type of reaction [32-35]. The reaction was performed in hexane at 32 °C, with the times indicated in Table 1.

The resulting sulcatyl acetate **3** and the remaining sulcatol **2** were purified by flash chromatography, and obtained in good yields (45 percent) and with excellent enantiomeric excess (*ee* > 99 percent). The enantiomeric excesses of the alcohol **2** and the acetate **3** were calculated from the chiral GC chromatograms (Fig. 1).

The stereochemical preference of CALB was determined by establishing the absolute configuration of the remaining sulcatol **2** and of the sulcatyl acetate **3**. The results obtained showed that the stereochemical preference of CALB for the sulcatol **2** was in accordance with the Kazlauskas rule [36]. By making a comparison between the obtained specific rotation values and those reported in the literature, sulcatol **2** was given the (*S*)-configuration [9,20,22,24], while the sulcatyl acetate **3** was assigned an (*R*)-configuration [26]. Figure 1 shows the chromatogram of sulcatyl acetate **3** obtained from the remaining sulcatol **2**. Racemics sulcatol **2** and sulcatyl acetate **3** demonstrated excellent enantioseparations in chiral column chromatography. The enzymatic reaction of racemic sulcatol **2** with CALB was conducted on a large scale (5.0 g, 39 mmol) and excellent performance in this enzymatic resolution was observed. The large scale enzymatic resolution produced (*S*)-**2**



Scheme 1. Synthesis of (±)-sulcatol **2** and (±)-sulcatyl acetate **3**

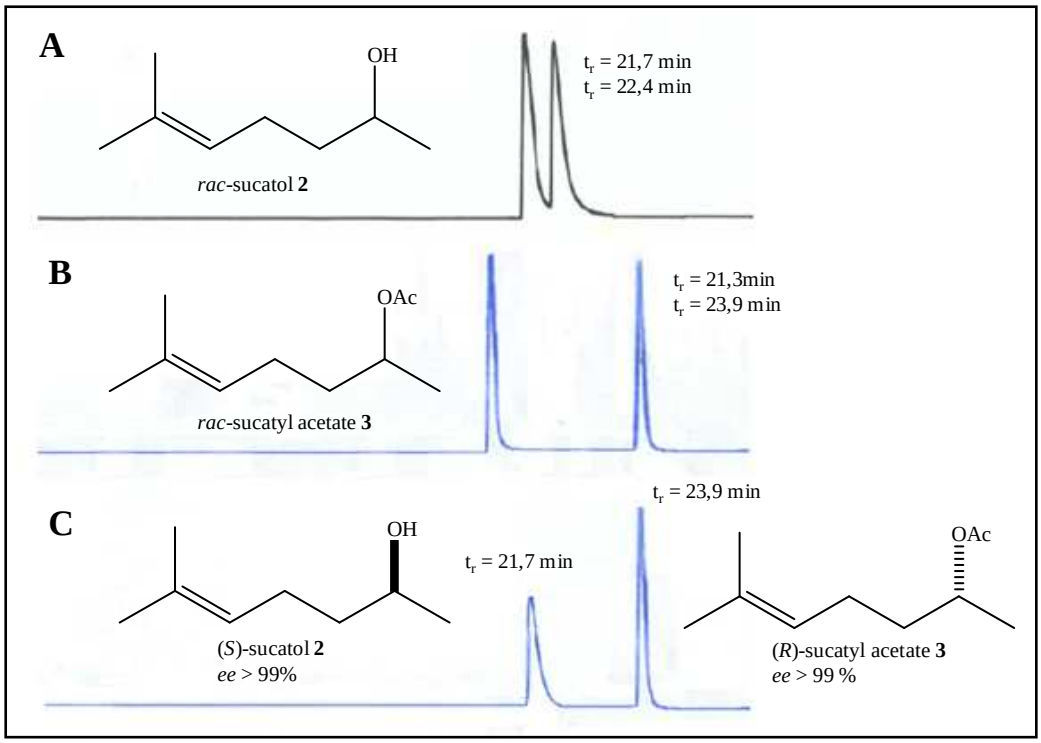


Fig. 1. Chromatograms: **A**. (±)-sulcatol **2**; **B**. (±)-sulcatyl acetate **3**; **C**. chemoenzymatic esterification of (±)-sulcatol **2** by CALB lipase in hexane (1 h).

and (*R*)-**3** compounds with high optical purities (*ee* > 99 percent), good yields (> 45 percent) and within a short time period (20 min to 1 h).

In addition, the enthalpy ($\Delta_R H^o_{298K}$) and Gibbs free energy ($\Delta_R G^o_{298K}$) of the acetylation reaction of sulcatol **2** were evaluated in the presence of vinyl acetate. We also did theoretical calculations to evaluate the thermodynamic parameters of the acetylation reaction of sulcatol **2** in the presence of vinyl acetate in the gas phase. Enthalpy (ΔH^o_{298k}) and Gibbs free energy (ΔG^o_{298k}) at 1 atm. were -14.83 and -14.59 kcal mol^{-1}, respectively. Our findings reveal that these values are negative, indicating that the process is exothermic and spontaneous, which is in agreement with what is observed experimentally.

CONCLUSIONS

We have prepared the optically active sulcatol **2**, as well as the respective sulcatyl acetate **3**, on a large scale (5 g, 39 mmol), with excellent enantiomeric excesses (*ee* > 99 percent) and high conversions rate. Lipase CALB proved to be highly efficient for the large scale resolution of the racemic sulcatol **2**. Thermodynamic parameters of the acetylation reaction of sulcatol **2** in the presence of vinyl acetate showed that ΔH^o_{298k} and ΔG^o_{298k} were negative. These results were in accordance with the chemoenzymatic esterifications for CALB.

ACKNOWLEDGMENTS

A.L.M. Porto wishes to thank FAPESP (Process No. 2006/54401-2) and CNPq (Process No. 472663/2004-6) for the financial support. H.V. Ferreira and L.C. Rocha wish to thank CNPq and CAPES, respectively, for the provision of scholarships. A.B.F. da Silva and R.B. Viana thank CNPq for the financial support and scholarships, respectively. The authors would also like to thank Novo Nordisk (Curitiba-

Paraná-Brazil) for the donation of the CALB enzyme (Novozyme 435).

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